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Patentanmeldung Nr. Patent application No. Demande de brevet n°

99112421.5

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Blatt 2 der Bescheinigung
Sheet 2 of the certificate
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NETHERLANDS ANTILLES

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TELEFAX COVER SHEET
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Istituto Farmacologico Serono SpA
Via Casilina, 125
00176 Rome - Italy
Phone: 39 06703841
Fax : 39 0670384756

To	European Patent Office D-80298 Munich - GERMANY
From	Daniele Pieraccioni
Date	30 June 1999
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Should Fax be Illegible/incomplete, please contact	Laura Petrucci 39-0670384635

Re:

New European Patent Application
In the name of: Applied Research Systems ARS Holding N.V.
Our ref: EP/407/Z "GRF Containing Lyophilized Pharmaceutical Composition"

Please find herewith enclosed:

DOCUMENTS	NUMBER OF PAGES
EPO FORM 1001	6
Description of the Patent Application	9
Claims of the Patent Application	2
Abstract of the Patent Application	1
General Authorization	1
EPO FORM 1010	1

TOTAL 20

Two additional copies of the above documents will follow with confirmation copy, by
courier.

GRF-CONTAINING LYOPHILIZED PHARMACEUTICAL COMPOSITIONS

FIELD OF THE INVENTION

The present invention concerns Growth Hormone Releasing Factor (GRF) containing pharmaceutical compositions. More precisely, it concerns compositions of saccharose-stabilized GRF.

BACKGROUND OF THE INVENTION

In the early 1980's several groups isolated and characterized growth hormone releasing factor (GRF).

GRF (also called Somatorelin) is a peptide secreted by the hypothalamus which acts on its receptor and can promote the release of growth hormone (GH) from the anterior pituitary. It exists as 44-, 40-, or 37-amino acid peptide; the 44-amino acids form may be converted physiologically into shorter forms. All three forms are reported to be active, the activity residing mainly in the first 29 amino acid residues. A synthetic peptide corresponding to the 1-29 amino acid sequence of human GRF [hGRF(1-29)], also called Sermorelin, has been prepared by recombinant DNA technology as described in European Patent EP 105 759.

Sermorelin has been used in the form of acetate for the diagnosis and treatment of growth hormone deficiency.

GRF has indeed a therapeutic value for the treatment of certain growth-hormone related disorders. The use of GRF to stimulate the release of GH is a physiological method in promoting long bone growth or protein anabolism.

It is well known that the natural form of GRF can suffer from chemical degradation in aqueous solution, primarily of Asn at position 8 which results in reduced biological potency

(Friedman, A.R. et al., *Int. J. Peptide. Protein Res.*, 37, 14-20, 1991; Bongers, J., et al., *Int. J. Peptide. Protein Res.* 39, 364-374, 1992).

The main hydrolytic reactions occurring in GRF are sensitive to pH and reported to be:
5 rearrangement of Asp³, at pH 4-6.5, cleavage of the Asp³-Ala⁴ bond at pH 2.5-4.5, deamidation and rearrangement of Asn⁸ at pH above 7 (Felix A.M. et al., *Peptides*, editors: Giralt E. and Andreu D., pp 732-733, Escom Publishers 1991). Due to the combined degradation pathways, unstabilized aqueous solutions GRF are most stable in the pH range 4-5. Bongers et al. (Bongers et al., 1992) have shown that the deamidation reaction at Asn⁸
10 increases rapidly as the pH is raised above pH 3.

WO 98/53844 describes stable liquid pharmaceutical compositions of hGRF containing nicotinamide and propylene glycol.

15 Various workers have made analogues of GRF by substitution of amino acids into the natural GRF sequence to improve the chemical stability (Serono Symposia USA, 1996; Friedman, 1991). While modification can be an effective means to improve the stability and retain bioactivity, it may be undesirable due to altered immunogenicity, which could be a problem for chronic therapies such as growth hormone deficiency.

20 According to EP 189 673 and US 4,963,529 (Sumitomo Pharma Inc.) GRF formulations can be prepared by lyophilization and stabilized by human serum albumin or glycine. JP 3083931 and EP 417 930 describe a GRF-containing nasal preparation which is rendered low-irritating to nasal mucosa by adding sodium chloride and/or sugar alcohols, such as
25 mannitol or sorbitol thereto.

In order that materials like hGRF be provided to health care personnel and patients, these materials must be prepared as pharmaceutical compositions. Such compositions must

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3

maintain activity for appropriate periods of time, must be acceptable in their own right to easy and rapid administration to humans, and must be readily manufacturable. In many cases pharmaceutical formulations are provided in frozen or in lyophilized form. In this case, the composition must be thawed or reconstituted prior to use. The frozen or lyophilized form is often used to maintain biochemical integrity and the bioactivity of the medicinal agent contained in the compositions under a wide variety of storage conditions, as it is recognized by those skilled in the art that lyophilized preparations often maintain activity better than their liquid counterparts. Such lyophilized preparations are reconstituted prior to use by the addition of suitable pharmaceutically acceptable diluent(s), such as sterile water for injection or sterile physiological saline solution, and the like.

Human GRF is found on the market in lyophilized formulations stabilized with mannitol GEREF®, Serono.

15 **DESCRIPTION OF THE INVENTION**

We have now found that saccharose confers a better stability to lyophilized formulations of hGRF.

20 The main object of the present invention is to provide pharmaceutical compositions comprising a solid intimate mixture of human GRF and a stabilizing amount of saccharose.

25 A further object is to provide a process for the preparation of said pharmaceutical composition, comprising the step of lyophilizing an aqueous solution of the components in the containers. Another object is to provide a presentation form of said pharmaceutical composition comprising the said solid mixture hermetically closed in a sterile condition within containers suitable for storage before use and suitable for reconstitution of the mixture for injectable substances. Such containers may be suitable for single dose administration or for multidose administration. Such lyophilized compositions also

preferably contain a bacteriostatic agent. The bacteriostatic agent is preferably m-cresol.

The lyophilized compositions of the invention may further comprise buffering agents.

5 Another object is to provide a solution of said solid mixture reconstituted into an injectable solution.

According to this invention the word "hGRF" is intended to cover any human GRF peptide, with particular reference to the 1-44, 1-40, 1-29 peptides and the corresponding amides 10 thereof (containing -NH₂ at their end). They are all commercial compounds. The preferred hGRF is hGRF(1-29)-NH₂.

The invention will now be described by means of the following Examples, which should not be construed as in any way limiting the present invention.

15

EXAMPLES

In order to evaluate the excipient's effect on the stability of the active ingredients, three 20 formulations of recombinant hGRF have been prepared with various excipients: saccharose, mannitol and mannitol/phosphate buffer. The filling volume was 2 ml. The compositions of the various formulations which were prepared are reported in Table 1.

Table 1

Formulation	hGRF (mg/ml)	Mannitol (mg/ml)	Saccharose (mg/ml)	Phosphoric Acid (mg/ml)	Sodium Hydroxide
1	5	18.2	-	-	-
2	5	18.2	-	0.98	q.s. to pH 4
3	5	-	34.2	-	-

The preparation of the lyophilizate was performed by dissolving the hGRF bulk powder in the solutions containing the stabilizers. The obtained solutions were filtered and filled into glass vials and lyophilized. The study of the stability of such formulations stored at 40°C and 50°C for 4 weeks, was performed by determinations of pH and peptide purity.

5

The chromatographic assay methodology (reverse phase HPLC) to evaluate the purity of hGRF was a gradient elution through a C-18 column, using a mobile phase (TFA/water/acetonitrile) at 1 ml/min and UV detection at 214 nm.

10 The pH was determined by a pHmeter on vials reconstituted with 5 ml of water for injection.

The results are summarized in Tables 2 and 3.

15

Table 2

pH

Formulation	pH					
	40°C			50°C		
	T=0	3 weeks	4 weeks	2 weeks	3 weeks	4 weeks
1	6.8	7.4	7.4	7.2	7.3	7.4
2	4.8	5.2	5.4	5.6	5.4	5.7
3	5.5	5.4	5.5	5.4	5.4	5.4

Table 3

Formulation	Peptide Purity (%)					
	40°C			50°C		
	T=0	3 weeks	4 weeks	2 weeks	3 weeks	4 weeks
1	97.7	96.3	95.7	93.7	92.9	91.8
2	97.7	95.6	94.8	89.4	88.5	84.2
3	97.8	97.9	97.8	97.8	97.8	97.6

Results showed that the formulation containing saccharose presented a better stability profile when compared to the formulations containing mannitol or mannitol/phosphate buffer.

10 Additional formulations having the composition of formulation 3 described in Table 1 were manufactured in different containers (vials); the composition is reported in Table 4.

Table 4

Formulation	hGRF (mg/vial)	Saccharose (mg/vial)
3a	3	20.5
3b	10	68.4

15 The formulations were stored at 5°C, 25°C and 40°C and tested for stability using the analytical methods described before (pH, purity and titre by RP).

Stability data have been generated up to 4 weeks; the results are reported in Tables 5 to 7.

Table 5

Formulation	pH			
		5°C	25°C	40°C
	T=0	4 weeks	4 weeks	4 weeks
3a	4.95	5.03	5.02	5.12
3b	4.96	5.09	5.06	5.13

Table 6

Formulation	Peptide purity (%)			
		5°C	25°C	40°C
	T=0	4 weeks	4 weeks	4 weeks
3a	97.7	97.7	97.5	97.4
3b	97.8	97.7	97.6	97.5

Table 7

Formulation	Peptide Content (mg/vial)			
		5°C	25°C	40°C
	T=0	4 weeks	4 weeks	4 weeks
3a	2.8	3.0	2.9	2.9
3b	9.8	9.5	9.8	9.8

10

The stability of reconstituted solutions with 1.5 and 5 ml 0.3% m-cresol at 5±3 °C and 25±2°C up to 1 month was also studied.

The stability data on the reconstituted solutions are reported in Tables 8 to 10.

Table 8

Formulation	Storage (°C)	pH				
		T=0	1 week	2 weeks	3 weeks	4 weeks
3a	5°C	4.94	5.03	5.04	5.05	5.18
3b	5°C	4.96	5.07	5.04	5.14	5.25
3a	25°C	4.94	5.05	5.07	5.07	5.19
3b	25°C	4.96	5.14	5.12	5.14	5.24

Table 9

5

Formulation	Storage (°C)	Peptide Purity (%)				
		T=0	1 week	2 weeks	3 weeks	4 weeks
3a	5°C	97.6	97.6	97.5	97.6	97.4
3b	5°C	97.6	97.5	97.4	97.5	97.4
3a	25°C	97.6	96.4	95.4	94.5	93.5
3b	25°C	97.6	96.3	95.4	94.7	93.5

Table 10

Formulation	Storage (°C)	Peptide Content (mg/vial)				
		T=0	1 week	2 weeks	3 weeks	4 weeks
3a	5°C	2.9	3.0	2.5	3.0	2.9
3b	5°C	9.6	10.0	9.1	10.0	9.9
3a	25°C	2.9	2.9	2.8	2.8	2.8
3b	25°C	9.6	10.0	9.3	9.5	9.4

EXAMPLE OF PHARMACEUTICAL MANUFACTURING

Materials: extra pure saccharose DAB, Ph Eur, BP, NF (Merck); water for injectables.

As containers have been used vials DIN 2R and DIN 6R (borosilicate glass type I) , rubber
5 closures (Pharmagummi W1816 V50) and aluminum rings and flip-off caps (Pharma-Metal
GmbH).

Preparation of hGRF solution containing saccharose: (for 200 vials containing each 3 or 10
mg hGRF).

10 Saccharose (17.1g) are dissolved into water for injectables (500 ml) in order to obtain the
starting saccharose solution.

The bulk of the hGRF (2 g) is added to the saccharose solution so as to obtain a final weight
of 400 g The solution is filtered through a 0,22 μ m Durapore sterile filter (Millipore).

15

Filling up and lyophilization

The vials are filled up with 0.6 and 2 ml of hGRF sterile solution , transferred to the freeze-
dryer and lyophilized according to the following cycle:

freezing: -25°C for 3 hrs

20 -15°C for 1 hr
-45°C for 3 hrs

- primary drying: -10°C for 13 hrs
- secondary drying: from -10°C to +40°C in 8 hrs; +40°C till end of cycle

CLAIMS

1. A pharmaceutical composition comprising a solid intimate mixture of human growth releasing factor (hGRF) and a stabilizing amount of saccharose, alone or in combination with other excipients.
5
2. The pharmaceutical composition according to Claim 1, wherein the solid intimate mixture is a lyophilizate.
- 10 3. The pharmaceutical composition according to any of Claims 1 to 2, wherein the stabilizing agent is saccharose alone.
4. The pharmaceutical composition according to any of Claims 1 to 3, containing 3 or 10 mg/vial of hGRF.
15
5. The pharmaceutical composition according to any of Claims 1 to 4 comprising 3 or 10 mg/vial of hGRF and 20.52 or 68.4 mg/vial of saccharose.
- 20 6. The pharmaceutical composition according to any of Claims 1 to 5 further comprising buffering agents.
7. A process for preparing a pharmaceutical composition according to any of Claims 1 to 6, comprising the preparation of an aqueous solution of the components, the distribution within containers and the lyophilization in the containers.
25
8. Forms of presentation of said pharmaceutical composition comprising the solid mixture according to any of Claims 1 to 6, hermetically closed in a sterile condition within a container suited for storage before use and for reconstitution of the mixture into a solvent or

11

into a solution for injectables.

9. A solution comprising the solid mixture according to any of Claims 1 to 6, reconstituted in a solvent or a solution for injectables.

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10. The solution according to any of Claim 9, wherein the pH is comprised between 4 and 6.

ABSTRACT

Human Growth Hormone Releasing Factor (GRF)-containing pharmaceutical compositions are described, more precisely, lyophilized compositions of hGRF stabilized by means of saccharose.

